Mammal Study 24: 35–41 (1999)

A karyological analysis of the Korean red-backed vole, *Eothenomys regulus* (Rodentia, Muridae), using differential staining methods

Masahiro A. Iwasa[1], Sang Hoon Han[2] and Hitoshi Suzuki[3]

[1,3]*Laboratory of Ecology and Genetics, Graduate School of Environmental Earth Science, Hokkaido University, Sapporo 060-0810, Japan*

[2]*Ecosystem Conservation Division, Ministry of Environment, 1, Joongang-dong, Kwacheon, Kyunggi-do 427-760, Korea*

Abstract. The conventional and G- and Q-banded karyotypes of the Korean red-backed vole *Eothenomys regulus* (2n=56) are described here for the first time. The autosomes were found to be composed of 26 pairs of acrocentrics and one pair of metacentrics, as in other species of red-backed voles. Side-by-side pair-matching analysis revealed that the G-banding patterns of *E. regulus* were essentially identical to those of the grey red-backed vole *Clethrionomys rufocanus*, and therefore the karyotype of *E. regulus* was of a "*rufocanus*" type, not of a "*glareolus*" type, which is characterized by 1–9 translocation. The sex chromosomes of *E. regulus* were found to be composed of a large subtelocentric X chromosome and a medium-sized subtelocentric Y chromosome, closely resembling those of *E. smithii* in both size and morphology. Both X and Y sex chromosomes were indistinguishable between these species, as far as conventional staining is concerned. Further analysis indicated, however, that *E. regulus'* Y chromosome has a large C-band area on the terminal half of its long arm, whereas *E. smithii* has a large C-band area on the proximal half of its long arm. Such C-band patterning implies the involvement of the Y chromosome in paracentric inversion during the course of speciation.

Key words: karyotype, *Eothenomys regulus*, the Korean red-backed vole, sex chromosomes.

The Korean red-backed vole was first described as *Craseomys regulus* by Thomas (1907), on the basis of the type specimen collected at Min-gyong, Korea. Now, this species is widely regarded as belonging to the genus *Eothenomys* (Corbet 1978; Kaneko 1990; Corbet and Hill 1991; Musser and Carleton 1993), although on the basis of molecular data from mitochondrial, and nuclear ribosomal DNA, its inclusion in the genus *Clethrionomys* has also been proposed (Wakana et al. 1996; Suzuki et al. 1999). Thus the taxonomic status of this vole remains uncertain. With the exception of the Korean species, the karyotypes of all of the East Asian red-backed vole species have been studied. Differential staining methods have shown that all of them share the same diploid number 2n=56 with essentially 26 pairs of

[1]*E-mail: masahiro@ees.hokudai.ac.jp*

[2]*Present address: Wildlife Information and Research Centre, Chongnyang, Tongdaemun-ku, Seoul 130-011, Korea*

acrocentrics (or subtelocentrics) and one pair of metacentrics, showing a high degree of karyotypic similarity (Tsuchiya 1981; Ando et al. 1988; Kashiwabara and Onoyama 1988; Yoshida et al. 1989; Sokolov et al. 1990; Obara et al. 1995; Kitahara and Harada 1996). Detailed G-banding analysis has revealed, however, that the red-backed vole species complex can be divided, karyologically, into two groups, the "*glareolus*" and the "*rufocanus*" groups (Gamperl 1982; Iwasa 1998). The "*glareolus*" group is characterized by the 1-9 translocation which can be seen in *C. glareolus*, *C. rutilus*, *C. gapperi* and *C. californicus* (Modi 1987; Modi and Gamperl 1989; Obara et al. 1995), whereas the "*rufocanus*" group (*C. rufocanus*, *C. rex* (dealt with as a synonym of *C. montanus*) and two Japanese species *E. andersoni* and *E. smithii*) shows no such translocation (Obara 1986; Ando et al. 1988; Kashiwabara and Onoyama 1988; Yoshida et al. 1989; Sokolov et al. 1990; Obara et al. 1995; Kitahara and Harada 1996).

The purpose of this study was to make the first examination of the karyotype of *E. regulus*, and to compare it with those of related species, so as to be able to ascertain, from a cytogenetic perspective, the phylogenetic position of *E. regulus* among the East Asian red-backed vole species.

Materials and methods

Two male *Eothenomys regulus* were captured, using Sherman live-traps, at Tonmyon-ri, Sesanmyon, Ponghwa-gun, Kyongsangbuk-do, Korea. They were identified on the basis of their cranial and dental characteristics as described by Kaneko (1990) (see Table 1 and Fig. 1), and preserved in 70% ethanol as specimens HEG22-97 and HEG49-97. Six

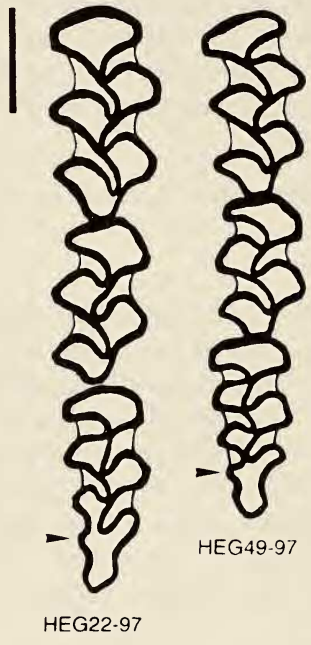

Fig. 1. Enamel patterns of the right upper molars of the *Eothenomys regulus* specimens examined in this study. (Arrowheads indicate the fourth outer small salient angle. Bar=1 mm. See Table 1 for specimen number).

Table 1. Morphological measurements of the Korean red-backed vole, *Eothenomys regulus*, examined in this study.

Specimen No.	Sex	Capturing date	T.L. (mm)	T. (mm)	T.R. (%)	H.F. (mm)
HEG22-97	male	25 Apr. 1997	142.5	36.0	33.8	19.3
HEG49-97	male	24 Apr. 1997	141.0	40.0	39.6	18.2

T.L.: Total length; T.: Tail length; T.R.: Tail rate; H.F.: Hind foot length.

Table 2. Number of cells observed.

Specimen No.	Conv. Giemsa	G-band	Q-band	C-band
HEG22-97	30	15	28	16
HEG49-97	62	36	115	22
total	92	51	143	38

Clethrionomys rufocanus collected in Hokkaido, and six *E. smithii* collected in Shikoku, were used for a comparison of the sex chromosomes.

Chromosome preparations were made from bone marrow cells after short-term culture (40 min at 37°C) in MEM containing 15% fetal calf serum and colchicine (final concentration 0.025 g/ml). The bone marrow cells were treated in 0.075 M KCl at 37°C for 18 min, followed by fixation with Carnoy's fixative (methanol: acetic acid = 3:1). Cell suspensions were dropped on slides and air-dried. Chromosomes were analyzed by both conventional and differential staining methods. For the latter staining method C-, Q- and G-bands were examined following methods described by Caspersson et al. (1971), Sumner et al. (1971) and Sumner (1972; see Table 2).

Results and discussion

Two red-backed vole specimens collected from the Korean Peninsula were examined intensively in order to determine their specific identification on the basis of their morphological features since two very similar species of voles, *E. regulus* and *C. rufocanus*, have been reported from the region (Corbet 1978). The two species closely resemble each other in morphology, but *E. regulus* has a specific "complex form" of enamel patterning on the upper third molar (Kaneko 1990), which is distinguishable from that of *C. rufocanus*. Our two specimens both had "complex form" upper third molars (Fig. 1), and so were confirmed as *E. regulus* (Kaneko 1990).

Eothenomys regulus was confirmed as having 26 pairs of acrocentrics and one pair of metacentrics, which was the smallest pair in the complement (Fig. 2a). The autosomes and the X chromosome (excluding its short arm) had G-banding patterns identical with those of *C. rufocanus* (Fig. 3) and other Japanese red-backed vole species. Thus, karyologically, *E. regulus* belongs to the "*rufocanus*" group, which has no 1–9 translocations (Gamperl 1982; Obara et al. 1995). As expected, the Q-banding patterns (Fig. 2b) were almost identical to the G-banding patterns (Fig. 3); the bright bands basically corresponded to G-positive bands. Centromeric regions showed dull fluorescence in all chromosomes after Q-banding.

In contrast to the highly consistent constitution of the autosomes of red-backed vole species, the sex chromosomes showed both inter- and intraspecific variation. The sex chro-

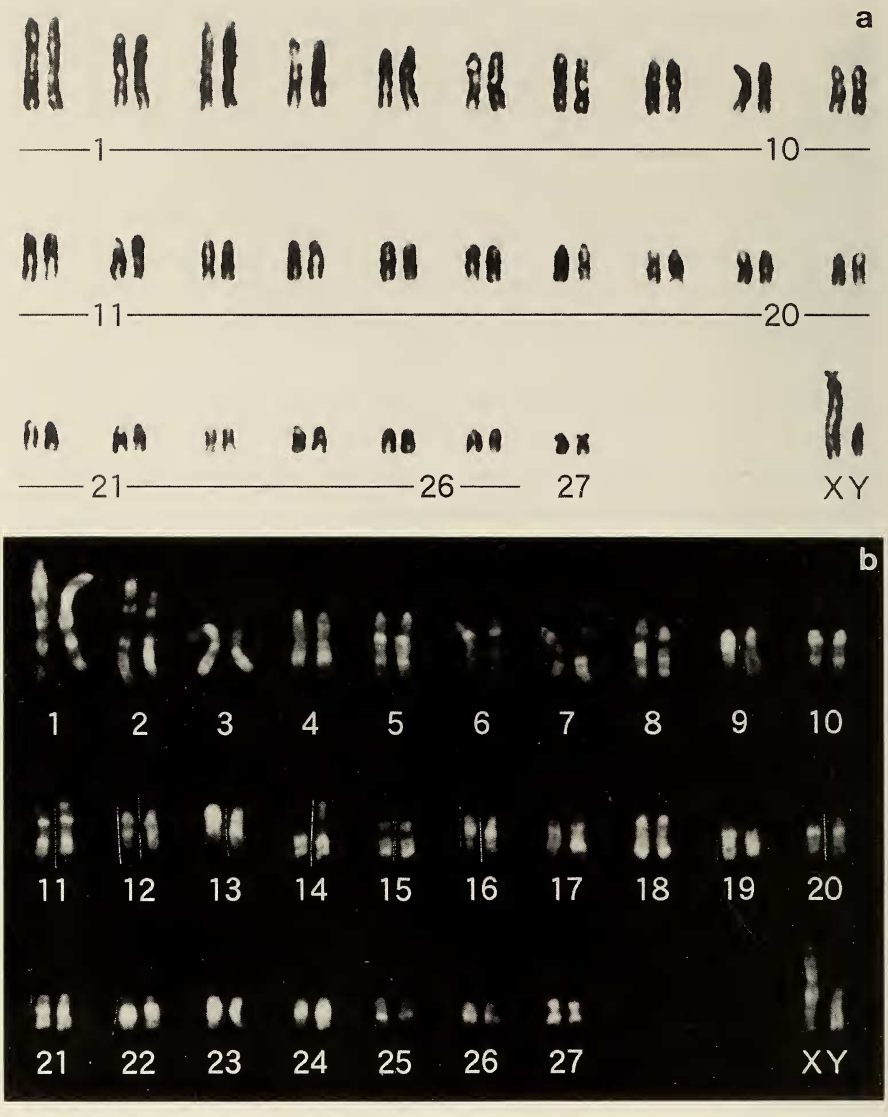

Fig. 2. Conventionally stained (a) and Q-banded (b) karyotypes of *Eothenomys regulus*.

mosomes of *E. regulus* proved to be composed of a large subtelocentric X and a medium-sized subtelocentric Y chromosome. Such a combination is markedly different from the XY chromosomes of *C. rufocanus*, which has a large acrocentric X and a small acrocentric Y chromosome (Fig. 4). Two distinct karyological forms of *E. smithii* have been reported, one with a small Y chromosome (the so-called *smithii* form of south-western Honshu and Shikoku (Fig. 4; Ando et al. 1988), and the other with a large Y chromosome the *kageus* form of central Honshu (Ando et al. 1988). The Y chromosome of *E. regulus* was equivalent in length to that of the small *smithii* form of *E. smithii*. A detailed comparison of the C-bands of *E. regulus* and *E. smithii* indicated, however, the possibility of a structural rear-

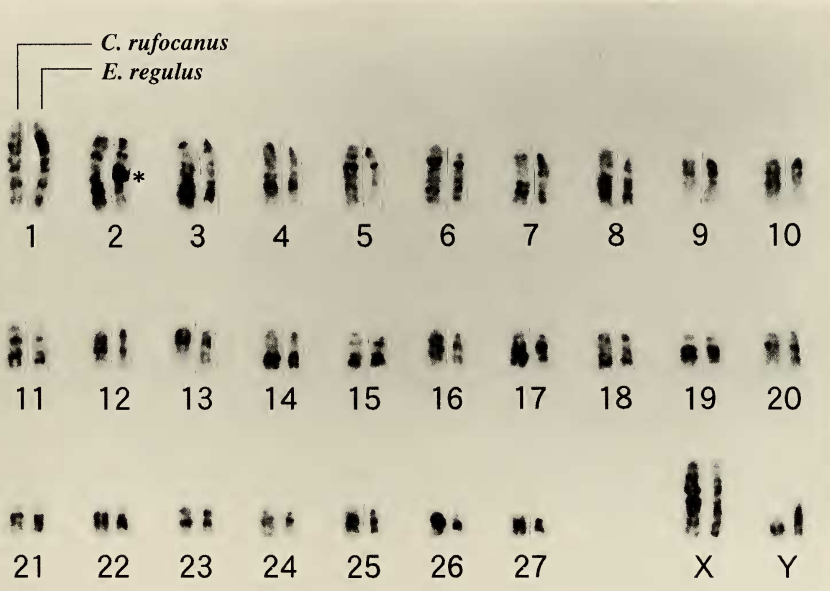

Fig. 3. Composite karyotype of *Clethrionomys rufocanus* and *Eothenomys regulus* prepared by side-by-side arrangement on the basis of G-band homology. (Left = *C. rufocanus*. Right = *E. regulus*. The asterisk indicates overlapping chromosomes).

rangement of the Y chromosome. The Y chromosome of *E. regulus* has a large C-band area on the terminal half of its long arm, whereas that of *E. smithii* has a similarly sized C-band on the proximal half of its long arm (Fig. 5). Similar C-band patterns in *E. smithii* have also been described by Ando et al. (1988) and Yoshida et al. (1989). Such interspecific differences in C-banding patterns can be explained by the occurrence of paracentric inversion involving most of the long arm of the Y chromosome during the course of speciation (Fig. 5).

The Y chromosome morphology suggests a closer relationship between *E. regulus* and *E. smithii* than with *C. rufocanus*, in which the Y chromosome is small and metacentric in the Primorskyi region of Russia, and small and acrocentric in Hokkaido, Japan (Vorontsov et

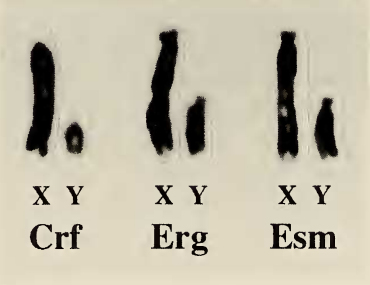

Fig. 4. Conventionally stained X and Y chromosomes of *Clethrionomys rufocanus* (Crf), *Eothenomys regulus* (Erg) and *E. smithii* (Esm).

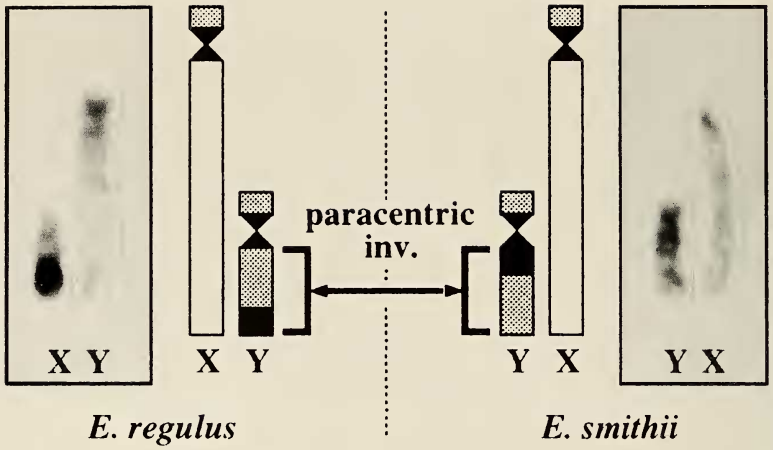

Fig. 5. C-banding patterns in the X and Y chromosomes of *Eothenomys regulus* and *E. smithii* shown by photographs and ideograms.

al. 1980; Tsuchiya 1981; Obara 1986; Yoshida et al. 1989). This chromosomal evidence is consistent with the fact that adult *E. regulus* and *E. smithii* both have rootless molars. In contrast, however, molecular phylogenetic data, on the variation of nuclear ribosomal and mitochondrial DNA, suggests a closer relationship between *E. regulus* and *C. rufocanus*, than with *E. smithii* (Wakana et al. 1996; Suzuki et al. 1999). The Shikoku *E. smithii* population, however, has specific mitochondrial sequences that differ from those of the Honshu and Kyushu populations, but which show affinities with those of both *C. rufocanus* and *E. regulus* (Suzuki et al. 1999). Thus, the phylogenetic relationships of these three red-backed voles, *E. regulus*, *E. smithii* and *C. rufocanus* are extremely complicated and as yet unresolved. Our cytogenetic and molecular findings indicate that interspecific genetic exchange may have played at least a partial role in complicating the genetic constitution of these three red-backed vole species. An analysis of the sequence variations in the genes specific to the X and Y chromosomes, and molecular cytogenetic analysis of the Y chromosomal C-heterochromatin, is considered likely to yield valuable information towards a more precise understanding of the phylogenetic relationships of these species. We are currently in the process of examining the relationships between the red-backed voles from these standpoints.

Acknowledgements: The authors are grateful to Dr. Yoshitaka Obara of Hirosaki University for his continuous encouragement throughout this study. We also thank Mr. C. C. Yoon for his kind co-operation in collecting animals at Tonmyon-ri, Kyongsangbuk-do, Korea. This study partly supported by a Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

References

Ando, A., Shiraishi, S., Harada, M. and Uchida, T. A. 1988. A karyological study of two intraspecific taxa in Japanese *Eothenomys* (Mammalia: Rodentia). Journal of the Mammalogical Society of Japan 13: 93–104.

Caspersson, T., Lomakka, G. and Zech, L. 1971. The 24 fluorescence patterns of the human metaphase chromosomes-distinguishing characters and variability. Hereditas 67: 89–102.

Corbet, G. B. 1978. The Mammals of the Palaearctic Region: a Taxonomic Review. Brit. Mus. (Nat. Hist.) & Cornell Univ. Press, London & Ithaca, 314 pp.

Corbet, G. B. and Hill, J. E. 1991. A World List of Mammalian Species, 3rd edn. Oxford University Press, Oxford, 243 pp.

Gamperl, R. 1982. Chromosomal evolution in the genus *Clethrionomys*. Genetica 57: 193–197.

Iwasa, M. A. 1998. Chromosomal and molecular variations in red-backed voles. Honyurui Kagaku [Mammalian Science] 38: 145–158 (in Japanese with English abstract).

Kaneko, Y. 1990. Identification and some morphological characters of *Clethrionomys rufocanus* and *Eothenomys regulus* from USSR, northeast China and Korea in comparison with *C. rufocanus* from Finland. Journal of the Mammalogical Society of Japan 14: 129–148.

Kashiwabara, S. and Onoyama, K. 1988. Karyotypes and G-banding patterns of the red-backed voles, *Clethrionomys montanus* and *C. rufocanus bedfordiae* (Rodentia, Microtinae). Journal of the Mammalogical Society of Japan 13: 33–41.

Kitahara, E. and Harada, M. 1996. Karyological identity of Anderson's red-backed voles from the Kii peninsula and central Honshu in Japan. Bulletin of the Forestry and Forest Products Research Institute No. 370: 21–30.

Modi, W. S. 1987. Phylogenetic analyses of chromosomal banding patterns among the Nearctic Arvicolidae (Mammalia: Rodentia). Systematic Zoology 36: 109–136.

Modi, W. S. and Gamperl, R. 1989. Chromosomal banding comparisons among American and European red-backed mice, genus *Clethrionomys*. Zeitschrift für Säugetierkunde 54: 141–152.

Musser, G. G. and Carleton, M. D. 1993. Family Muridae. In (D. E. Wilson and D. A. Reeder, eds.) Mammal Species of the World. A Taxonomic and Geographic Reference. 2nd edn. Pp. 501–755. Smithsonian Institution Press, Washington and London.

Obara, Y. 1986. G-band homology between the Japanese red-backed vole, *Clethrionomys a. andersoni* and the grey red-backed vole, *C. rufocanus*. Chromosome Information Service 40: 7–9.

Obara, Y., Kusakabe, H., Miyakoshi, K. and Kawada, S. 1995. Revised karyotypes of the Japanese northern red-backed vole, *Clethrionomys rutilus mikado*. Journal of the Mammalogical Society of Japan 20: 125–133.

Sokolov, V. Ye., Aniskin, V. M. and Serbenyuk, M. A. 1990. Comparative cytogenetics of 6 species of the genus *Clethrionomys* (Rodentia, Microtinae). Zoologicheskii Zhurnal 69: 145–151 (in Russian with English summary).

Sumner, A. T. 1972. A simple technique for demonstrating centromeric heterochromatin. Experimental Cell Research 75: 304–306.

Sumner, A. T., Evans, H. J. and Buckland, R. A. 1971. New technique for distinguishing between human chromosomes. Nature New Biology 232: 31–32.

Suzuki, H., Iwasa, M., Harada, M., Wakana, S., Sakaizumi, M., Han, S. H., Kitahara, E., Kimura, Y., Kartavtseva, I. V. and Tsuchiya, K. 1999. Molecular phylogeny of red-backed voles in Far East Asia based on variation in ribosomal and mitochondrial DNA. Journal of Mammalogy 80: 512–521.

Thomas, O. 1907. The Duke of Bedford's zoological exploration in eastern Asia. II. List of small mammals from Korea and Quelpart. Proceedings of Zoological Society of London, 1906: 858–865.

Tsuchiya, K. 1981. On the chromosome variations in Japanese cricetid and murid rodents. Honyurui Kagaku [Mammalian Science] 42: 51–58 (in Japanese).

Vorontsov, N. N., Lyapunova, E. A., Borissov, Y. M. and Dovgal, V. E. 1980. Variability of sex chromosomes in mammals. Genetica 52/53: 361–372.

Yoshida, I., Obara, Y. and Matsuoka, N. 1989. Phylogenetic relationships among seven taxa of the Japanese microtine voles revealed by karyological and biochemical techniques. Zoological Science 6: 409–420.

Wakana, S., Sakaizumi, M., Tsuchiya, K., Asakawa, M., Han, S. H., Nakata, K. and Suzuki, H. 1996. Phylogenetic implications of variations in rDNA and mtDNA in red-backed voles collected in Hokkaido, Japan, and in Korea. Mammal Study 21:15 –25.

Received 17 February 1999. Accepted 20 May 1999.